METHOD OF INCREASING THE TRANSGENE-CODED
BIOMOLECULE CONTENT IN ORGANISMS

The present invention relates to a method of increasing the transgene-coded biomolecule content in organisms, in particular in plants. The method is based on a change in the distribution of ATP and/or ADP in cells of the organism which can be carried out by various procedures.

Both naturally occurring and synthetic proteins, peptides and nucleic acids have highly interesting properties as regards to their uses as active substances and materials, but are often only available in very small amounts. Since it is also often not possible to obtain them efficiently under economic conditions and in sufficient amounts in recombinant host systems, e.g. in bacteria, such as *Escherichia coli*, *Bacillus subtilis*, *etc.*, commercial use cannot be realized. In order to be able to obtain more complex proteins and peptides or nucleic acids which are difficult to produce or cannot be produced at all in lower organisms, cells of higher organisms having an inherent complex protein and nucleic acid biosynthesis machinery are increasingly required as host cells. Transgenic animals, plants, mosses, algae, *etc.* have offered themselves as new recombinant hosts for some years now. Due to the availability of increasing numbers of well characterized biomolecules from molecular research, use of such hosts is gaining in importance for their production.

However, in host organisms the content of transgene-coded biomolecules does not always lie within the desired range. In particular the yields obtained in the host cells for the production of proteins or nucleic acids on an industrial scale are insufficient. On the one hand, this is due to the regulation of gene expression and, on the other hand, also caused by a degradation of the transgenic products by the host organism. In general, an increase in the expression and an increase in the amount of biomolecules stored in the organism is desirable for increasing the content of transgenic proteins, peptides and nucleic acids. This could raise the efficiency of the production of biomolecules in transgenic organisms and subsequently facilitate their recovery

and purification.

In order to obtain a high content of transgene-coded biomolecules, it is necessary to make use of those regulation mechanisms resulting in an increase of expression and to avoid or eliminate those suppressing the production or degrading the products. The use of strong promoters is a general approach for increasing transcription and thus raising the amount of mRNA made. This is usually also accompanied by an increase in the amount of foreign protein formed.

In order to protect once formed mRNA from an increased turnover, which plays a role in gene silencing, all measures preventing detection of RNA as foreign are suited. Such measures are e.g. the prevention of double-stranded RNA formation, the adaptation of the GC content to that of the host cell and the use of repressor proteins for suppressing post-translational gene silencing (De Wilde, Plant Molecular Biology **545** (2000), 347-359). By adapting the codon usage to that of the host cell it is possible to achieve an increase in translation. The transgene-coded biomolecule content can also be raised by lowering the formation of an endogenous storage protein, as accomplished by Goossens *et al.*, (FEBS Letters **456** (1999), 160-164) by means of the antisense technology. Another possibility of raising the foreign protein expression in transgenic organisms is the construction of fusion proteins between the target proteins and e.g. chaperonins or chaperonin binding domains.

However, it has only been possible thus far to raise the content of the desired transgenic molecules in organisms to some degree by these methods. In order to render the production of biomolecules in transgenic organisms more efficient, a mechanism is highly required which can be used as such or also in addition to said methods and in this connection raises the transgenically encoded biomolecule content significantly.

The present invention is thus based on the technical problem of providing a means by which an increase in the transgenic biomolecule content can be achieved in organisms, in particular in plants.

This technical problem is solved by the subject matters defined in the claims. The present invention comprises a novel mechanism of increasing the transgene-coded biomolecule content in organisms, such as plants, which is based on influencing the energy metabolism of the cells. It has been found surprisingly that a physiological change can be caused by modifying the distribution of ATP or ADP in the cell so as to achieve a significantly higher content of transgenically coded products in cells of the organism.

ATP is the universal energy carrier of all live cells. Energy in the form of ATP is required for almost all anabolic pathways. In heterotrophic plant cells, ATP is mainly synthesized by means of oxidative phosphorylation in the mitochondria from ADP and inorganic phosphate. Under anaerobic conditions, this is done by means of substrate-level phosphorylation in the cytosol. ATP is transported out of the mitochondria via the mitochondrial ADP/ATP transport protein which is one of the best studied membrane proteins. The mitochondrial ADP/ATP transport protein catalyzes exclusively the ATP export in return for the import of ADP.

In the case of heterotrophic vegetable storage tissues a comparatively large amount of ATP is taken up into the storage plastids to energize biosynthesis steps only occurring there, such as starch or fatty acid biosynthesis. This uptake is catalyzed by a plastidiary ATP/ADP transport protein which is localized in the inner coat membrane and enables the ATP uptake in return for the ADP release.

In order to analyze the effect of modified plastidiary ATP/ADP transporter activities on the carbohydrate balance, transgenic potato plants having increased or reduced transporter activity were produced in the experiments resulting in the present invention.

The amount of endogenous plastidiary ATP/ADP transporter in potatoes (AATP1, *Solanum tuberosum* St) was reduced by means of antisense inhibition. Part of the AATP1,St-coding cDNA was introduced in antisense orientation into the potato genome. This cDNA was

controlled by the constitutive CaMV 35S promoter. Various independent lines having in each case individually reduced activity of the plastidiary ATP/ADP transporter being obtained. The activity of the plastidiary ATP/ADP transporter was thus reduced to 64 % to 79 % as compared to that of non-transgenic control plants. The transgenic potato plants showed no phenotypic changes in the region of the aboveground green tissues. In contrast thereto, the morphology of the tubers was markedly altered (branched tubers) and the starch content dropped to about 50 % as compared to the non-transgenic control plants (Tjaden *et al.*, Plant Journal, **16** (1998), 531-540). Summarized, due to the reduced ATP/ADP transporter activity comparatively less ATP was taken up into the plastids and consequently less starch was produced.

Furthermore, transgenic potato plants having an increased activity of the plastidiary ATP/ADP transporter were produced by introducing the cDNA for the plastidiary ATP/ADP transporter from *Arabidopsis thaliana* (AATP1,AT) in sense orientation into the potato genome under the control of the 35S promoter. This led to various independent lines each having individually increased activity of the plastidiary ATP/ADP transporter. The measured activity of the plastidiary ATP/ADP transporter was between 130 and 148 % in the various lines as compared to those in non-transgenic control plants. The transgenic potato plants showed no phenotypic changes in the region of the aboveground green tissues. However, the starch content in the tubers was increased by up to about 150 % as compared to the control (Tjaden *et al.*, *supra*). Summarized, due to the increased ATP/ADP transporter activity comparatively more ATP was taken up into the plastids and therefore more starch was produced.

There is reason to suppose that the change in the ATP or ADP concentrations in certain parts of a plant cell has considerable effects on the cell metabolism and the regulation of genes. It was thus investigated in the studies conducted in connection with the present invention whether such a change also influences the protein content in the plant cells. For this purpose, transgenic potato plants of the Desirée variety were produced e.g. by means of the gene constructs described in Tjaden *et al.* (*supra*) to either reduce via "antisense" or increase via "sense" constructs the ATP/ADP transporter activity. The resulting transgenic plants were partially hypertransformed

using another transgene. The plants obtained were subjected to a proteinchemical analysis, and the content of different foreign proteins and whole protein in tuber extracts was determined. It turned out that there was a marked increase in the foreign protein content in transgenic plants (*cf.* below Examples 1-2).

Thus, the present invention relates to a method of increasing the transgene-coded biomolecule content in organisms, prefeably in plants, which is characterized by changing the distribution of ATP and/or ADP in cells of the organisms (as compared to the original situation).

An increase in the content of transgene-coded biomolecules is understood to mean every increase in the concentration of said biomolecules in an extract obtained from the tissues of the transgenic organisms as compared to the content in an extract obtained from organisms which do not show the change in the distribution of ATP and/or ADP in the cells according to the present invention. For example, this increase can be effected by an enhanced accumulation of the biomolecules in one or more cell compartments, such as the endoplasmic reticulum, the plastids, the vacuoles, the lysosomes, the mitochondria, the cell nucleus, the Golgi apparatus, the peroxisomes, the cytosol and others. In a preferred embodiment of the described method, the content of transgene-coded biomolecules is raised selectively, the content of endogenous biomolecules in cells of the organisms being not changed significantly. This increase can be constitutive or regulated temporally, locally or inducibly.

Within the meaning according to the present invention the transgene-coded biomolecules relate to both proteins and peptides as well as to nucleic acid molecules. In particular peptides, proteins and nucleic acids which are not expressed naturally in the respective target organism are mentioned. However, peptides, proteins and nucleic acids which are not expressed naturally in their present form are also transgene-coded biomolecules. They include e.g. all forms of modified or non-native proteins, peptides and nucleic acids such as hybrid proteins, chimeric proteins and chimeric nucleic acid constructs. Fragments of proteins, peptides or nucleic acids also represent transgenic biomolecules within the meaning according to the present invention. In

particular proteins, peptides or nucleic acids whose expression pattern was modified in the respective host organism shall also be transgenically encoded biomolecules within the meaning according to the invention. Examples thereof are proteins, peptides and nucleic acids whose expression is not regulated naturally in the present form. For example, the natural expression thereof can be regulated temporally, locally or inducibly in another way. In particular, the change of the expression of the sequences coding for the biomolecules can also show as an increase or reduction of the expression rate. Nucleic acids are in particular all deoxyribonucleic acids and ribonucleic acids. They are preferably available as ribozymes, single-stranded or double-stranded oligonucleotides or also as relatively long-chain nucleic acid molecules. Particularly preferred proteins are antibodies, aptamers, receptors, enzymes, growth factors, hormones and specific antigen molecules for use in diagnosis, therapy and the prevention of diseases, such as viral diseases or cancerous diseases. Such proteins may be e.g. interferons, immunoglobulins, growth hormones, insulin, collagen, plasminogen activator, blood factors such as factors I to XII, histocompatibility antigens, enzymes, tumor marker proteins and antibodies specific thereto as well as viral antigens and antibodies specific thereto.

The organisms suitable for use in the method according to the invention may contain one or more transgenes and express them in parallel or sequentially. The parallel expression of several transgenes is conceivable via the control of the coding sequences by constitutive and/or inducible promoters. A sequential expression can be achieved by the regulation of the gene expression of several transgenes in an organism, which can be induced in different ways.

The organisms suitable for the method according to the invention are animals, humans and plants. The term "animals" as used herein comprises preferably mammals, e.g. cows, horses, goats, sheeps, pigs, mice, rats and rabbits. The plants may, in principle, be plants of any species, i.e. both monocotyledonous and dicotyledonous plants. The term "plants" as used herein comprises preferably gramineae, chenopodiacea, leguminousea, brassicaceae, solanaceae, fungi, mosses, and algae. Crop plants, e.g. plants such a wheat, barley, rice, corn, sugar beets, sugarcane, rape, mustard, oilseed rape, flax, safflower, peas, beans, lupins, tobacco, lucerne,

soya, bananas, ananas, potatoes, sunflowers, melons, sweet potatoes, spelt, alfalfa, paprika, topinambur, tomatoes, durum wheat or rye are particularly preferred.

In a preferred embodiment, the method according to the invention is characterized in that the activity or concentration of a protein involved in the subcellular distribution of ATP and ADP is increased or reduced in the organism. This protein is usually a protein which is naturally available in the corresponding organism, e.g. the mitochondrial ADP/ATP transport protein, the plastidiary ATP/ADP transporter or the plastidiary triose phosphate/phosphate transporter. A particularly preferred embodiment of the method according to the invention is one in which the expression of a gene which codes for a protein involved in the subcellular distribution of ATP and ADP is increased or reduced. This gene expression can be modified by methods known to a person skilled in the art. For example, this can be effected by the above changes in the protein concentration and those described in the examples using antisense or sense constructs. A change in the protein activity or concentration can basically be effected via both gene expression and a functional inhibition of the protein activity, e.g. by the expression of binding, inhibiing, neutralizing or catalytic antibodies or other specifically binding and blocking proteins or peptides, by ribozymes, single-stranded or double-stranded oligonucleotides, aptamers, lipids, natural receptors, lectins, carbohydrates, *etc.*

In the method according to the invention the ATP or ADP concentration in cell compartments can also be influenced by introducing a protein (polypeptide) which is not naturally available in the respective organism. In order to obtain the localization of the protein in the desired cell compartment it may be favorable for the protein to have a signal peptide, so that it can be transported into certain cell compartments of a plant cell. The person skilled in the art is familiar with suitable signal peptides and methods of linking the signal peptides with a desired protein. For example, reference is made to the signal peptide of amylase from barley as to the apoplast (Düring *et al.*, Plant Journal 3 (1993), 587-598), to a murine signal peptide, to the combination or murine signal peptide and the KDEL-ER retention signal as regards ER (Artsaenko *et al.*, Molecular Breeding 4 (1998), 313-319), to the targeting signal of a mammal-alpha-2,5-

sialyltransferase regarding the Golgi apparatus (Wee *et al.*, Plant Cell IV (1998), 1759-1768), to the vacuolar localizing signal of a vacuolar chitinase from cucumber as regards the vacuoles (Neuhaus *et al.*, Proc. Natl. Acad. Sci. U.S.A. **88** (1991), 10362-10366), to the ferredoxin transit peptide as to the chloroplasts and plastids, and to the transit peptide of tryptophanyl tRNA synthethase from yeast regarding the mitochondria (Schmitz and Lonsdale, Plant Cell 1 (1998), 783-791). Basically, the protein involved in the subcellular distribution of ATP and APD can be administered by various methods, e.g. via media, such as the culture media, of a plant or of parts thereof, in particular plant cells. However, as pointed out above already, it is preferred to administer the protein to plants or parts thereof in the form of a nucleic acid coding for it, e.g. DNA or RNA. For this purpose, it is necessary for the nucleic acid to be available in an expression vector or to be ligated with sequences thereof. In this connection, it can be favorable for this vector or these sequences to enable an expression of the nucleic acid in cell compartments. Such expression vectors or sequences are known to the person skilled in the art. For example, reference is made to Svab *et al.*, Proc. Natl. Acad. Sci. U.S.A. **87** (1990), 8526-8530; Khan and Maliga, Nature Biotechnology **17** (1999), 910-915; and Sidorov *et al.*, Plant Journal **19** (1999), 209-216.

Methods of constructing the expression vectors containing the desired gene, e.g. for a plastidiary ATP/ADP transporter from *Arabidopsis thaliana* (AATP1,At) in expressible form are known to the person skilled in the art and also described in common standard works (*cf.* e.g. Sambrook *et al.*, 1989, Molecular Cloning, A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). The expression vectors can be based on a plasmid, cosmid, virus, bacteriophage or another vector common in genetic engineering. These vectors may have further functional units which effect stabilization of the vector in the plants, for example. If used for plants they may contain left-border and right-border sequences of agrobacterial T-DNA so as to enable stable integration into the genotype of plants. A termination sequence may also be present which serves for the correct termination of transcription and the addition of a poly-A sequence to the transcript. Such elements are described in the literature (cf. Gielen *et al.*, EMBO J. **8** (1989), 23-29) and can be exchanged as desired.

The person skilled in the art is familiar with suitable promoters for the expression of the gene coding for the desired protein. The promoters include e.g. the cauliflower mosaic virus 35S promoter (Odell *et al.*, Nature **313** (1995), 810-812), the *Agrobacterium tumefaciens* nopaline synthase promoter and the mannopine synthase promoter (Harpster *et al.*, Molecular and General Genetics **212** (1988), 182-190).

The increase or decrease of the above-described protein activities can be effected constitutively or temporally, locally or be induced by certain stimuli. A temporally or locally limited or inducible increase or decrease of the protein activities also suppresses the changes in the tuber morphology, described by Tjaden *et al.* (*supra*).

Thus, another preferred embodiment of the method according to the invention is characterized in that the expression of the gene whose product causes a change in the distribution of ATP and/or ADP in cells of the organism is regulated in the organism temporally, locally or inducibly. For example, the gene coding for the desired protein can be linked with an inducible promoter, which permits e.g. the control of the synthesis of the desired protein, e.g. in a plant, at a desired time. Suitable promoters are known to the person skilled in the art and they comprise e.g. the anaerobically inducible Gap C4 promoter from corn (Bülow *et al.*, Molecular Plant-Microbe Interactions **12** (1999), 182-188), PR promoters such as L-phenylalanine ammonium lyase, chalcone synthase and hydroxyproline rich glycoprotein promoters, inducible by ethylene (Ecker and Davies, Proc. Natl. Acad. Sci. U.S.A. (1987), 5202-5210) and a dexamethasone-inducible chimeric transcription induction system (Kunkel *et al.*, Nature Biotechnology **17** (1990), 916-918), the IncW promoter from corn inducible by saccharose or D-glucose (Chen *et al.*, Proc. Natl. Acad. Sci. U.S.A. **96** (1999), 10512-10517). Reference is also made to Dalta *et al.*, Biotechnology Annual Review **3** (1997), 269-290, and Gatz and Denk, Trends in Plant Science **3** (1998), 352-358. Furthermore, promoters are suited which permit local regulation of the expression, i.e. only in certain plant parts or organs. Such promoters are e.g. the patatin promoter from potato (Liu *et al.*, Molecular and General Genetics **223** (1990), 401-406) (tuber-

specific), the napin promoter from allseed rape (Radke *et al.*, Theoretical and Applied Genetics 75 (1988), 685-694) (embryo-specific in the seed), the RolC promoter from *Agrobacterium rhizogenes* (Yokoyama *et al.*, Molecular and General Genetics **244** (1994), 15-22) (phloem-specific), the TA29 promoter from tobacco (Kriete *et al.*, Plant Journal **9** (1996), 809-818) (tapetum-specific), the LeB4 promoter from *Vicia faba* (Bäumlein *et al.*, Molecular and General Genetics **225** (1991), 121-128) (seed-specific) and the rbcS and cab promoters from petunia (Jones *et al.*, Molecular and General Genetics **212** (1988), 536-542) (leaf-specific or limited to photosynthetically active tissues).

In another preferred embodiment of the method according to the invention the expression of the plastidiary ATP/ADP transporter is raised or lowered. In this connection, the expression can be lowered by introducing an antisense construct suppressing the expression of the endogenous gene, and the expression can be raised by introducing a sense construct. The sense construct may be a gene available on an expression vector for the endogenous transporter e.g. under the control of a strong promoter but also a heterologous gene which codes for a transporter from another organism, preferably a closely related organism.

A large number of cloning vectors which contain a replication signal for *E. coli* and a marker gene for the selection of transformed bacterial cells are available for the production of the expression vectors which shall be introduced into plants. Examples of such vectors are pBR322, pUC series, M13mp series, pA-CYC184, *etc*. The desired sequence may be introduced into the vector at an appropriate restriction site. The resulting vector is used for the transformation of *E. coli* cells. Transformed *E. coli* cells are cultured in a suitable medium, then harvested and lysed. The vector is then recovered. In general, restriction analyses, gel electrophoresis and further biochemical and molecular-biological methods are used as analytical methods for characterizing the vector DNA obtained. The vector DNA can be cleaved after every manipulation and the DNA fragments obtained can be linked with other DNA sequences. Each vector DNA sequence can be cloned into the same or into other vectors.

A number of methods are available for the introduction of the above expression vectors into a plant cell. These methods comprise transformation of plant cells with T-DNA using *Agro - bacterium tumefaciens* or *Agrobacterium rhizogenes* as trans formation means, fusion of protoplasts, injection, electropo ration of DNA, introduction of DNA using the biolistic method and further possibilities.

The injection and electroporation of DNA in plant cells do generally not make special demands on the vectors used. It is possible to use simple plasmids such as pUC derivatives. However, if whole plants shall be regenerated from cells transformed in this way, a selectable marker should be present. Suitable selectable markers are known to the person skilled in the art and they comprise e.g. the neomycin phosphotransferase II gene from *E. coli* (Beck *et al.*, Gene **19** (1982), 327-336), the sulfonamide resistance gene (EP-369637), and the hygromycin resistance gene (EP-186425). Depending on the method of introducing the desired gene into the plant cell, further DNA sequences may be required. For example, if the Ti or Ri plasmid is used for the transformation of the plant cell, at least the right border, but often the right and left borders, of the Ti and Ri plasmid T-DNA must be connected as a flanking region with the genes to be introduced.

If agrobacteria are used for the transformation, the DNA to be introduced must be cloned into special vectors, i.e. into either an intermediary vector or a binary vector (*cf.* the below examples). Due to sequences homologous to sequences in the T-DNA, the intermediary vectors can be integrated into the Ti or Ri plasmid of the agrobacteria by homologous recombination. It also contains the vir region necessary for the T-DNA transfer. Intermediary vectors cannot replicate in agrobacteria. By means of a helper plasmid, the intermediary vector can be transferred to *Agrobacterium tumefaciens*. Binary vectors can replicate in both *E. coli* and *Agrobacterium*. They contain a selection marker gene and a linker or polylinker, which are surrounded by the right and left T-DNA border. They can be transformed directly into the agrobacteria. The agrobacterium serving as a host cell should contain a plasmid which carries a vir region. The vir region is necessary for the transfer of T-DNA into the plant cell. Additional T-DNA may be

present. The agrobacterium transformed in this way is used for the transformation of plant cells.

In order to transfer the DNA into the plant cell, plant explants can usefully be cocultured with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*. Whole plants can then be regenerated again from the infected plant material (e.g. leaf portions, stem segments, roots, but also protoplasts or suspension-cultured plant cells) in a suitable medium which may contain antibiotics or biocides for the selection of transformed cells. The resulting plants can subsequently be studied for the presence of the introduced DNA. Alternative systems for the transformation of monocotyledonous plants are transformation by means of a biolistic approach, electrically or chemically induced DNA uptake into protoplasts, electroporation of partially permeabilized cells, macroinjection of DNA into inflorescences, microinjection of DNA into microspores, oocytes and pro-embryos, DNA uptake by germinating pollens, and DNA uptake into embryos by swelling (for an overview see Potrykus, Biotechnologie **8** (1990), 535-542). While the transformation of dicotyledonous plants is well established via Ti plasmid vector systems using *Agrobacterium tumefaciens*, more recent studies indicate that monocotyledonous plants are also very well accessible to transformation by means of vectors based on Agrobacterium.

In a preferred embodiment, the expression vectors used according to the invention contain localization signals for localization in cell compartments, in particular in endoplasmic reticulum (ER), apoplasts, Golgi apparatus, plastids, peroxisomes, mitochondria and/or vacuoles. Reference is made to the above statements on the signal peptides. The KDEL-ER targeting peptide, the Golgi localization signal of ß-1,2-N-acetylglucosamine transferase (Gntl), the transit peptide from the small subunit of ribulose bisphosphate carboxylase and/or the vacuolary targeting signal SKNPIN are particularly preferred as localization signals.

In principle, the plant portions desired for the expression of the transgene relate to any plant part, in any case to replication material of these plants, e.g. seeds, tubers or bulbs, rootstocks, seedlings, cuttings, *etc.*

In principle, the present invention also enables an increase in the expression of transgenes in animals and humans. For this purpose, the above protein can be administered as such or in combination with a signal peptide to animals, humans or cells thereof. Such a signal peptide can be e.g. a murine signal peptide, a combination of a murine signal peptide and the KDEL-ER retention signal, or the targeting signal of a mammalian alpha-2,6-sialyltransferase as regards the Golgi apparatus. Furthermore, the protein can be administered in the form of a nucleic acid coding for it, e.g. DNA or RNA, to animals, humans or cells thereof. Administration in the form of a nucleic acid requires that the latter is present in an expression vector or is ligated with sequences thereof. Reference is made to the above general statements on expression vectors and their production. In addition, reference is made to vectors which are suited for the gene therapy in animals and humans. In them, the nucleic acid can be controlled by an inducible or tissue-specific promoter, such as metallothionein I or polyhedrin promoter. Preferred vectors are e.g. viruses, such as retroviruses, adenoviruses, adeno-associated viruses or vaccinia viruses. Examples of retroviruses are MoMuLV, HaMuSV, MUMTV, RSV or GaLV. Furthermore, the nucleic acid coding for the polypeptide can be transported to the target cells in the form of colloidal dispersions. They comprise e.g. liposomes and lipoplexes (Mannino *et al.*, Bio-techniques **6** (1988), 682).

According to the invention, the above protein is administered to humans and cells. In principle, the animals may belong to any animal species. They are preferably useful and domestic animals, e.g. cattle, horses, sheep, pigs, goats, chickens, turkeys, dogs, cats, *etc.*

Examples of transgenes whose expression in animals and humans can be raised are in particular peptides, proteins and nucleic acids. The particularly preferred proteins are antibodies, aptamers, receptors, enzymes, growth factors, hormones and specific antigen and antibody molecules for use in diagnosis, therapy and the prevention of both viral diseases and cancerous diseases. Such proteins are e.g. interferons, immunoglobulins, growth hormones, insulin, collagen, plasminogen activator, blood factors such as factors I to XII, histocompatibility antigens, enzymes, tumor

marker proteins and antibodies specific thereto as well as viral antigens and antibodies specific thereto. Examples of nucleic acids are single-stranded and double-stranded RNA or DNA, oligonucleotides and ribozymes.

Brief description of the figures:

Figure 1

Figure 1 shows the NptII protein content in ng/ml in the extract of potato tubers. The lines MBP7sATPT contain the sense gene construct for the plastidiary ATP/ADP translocator from *Arabidopsis thaliana* in transgenic potato plants of the Désirée variety. The lines MPB/aATPT contain the antisense gene construct for the plastidiary ATP/ADP translocator from *Arabidopsis thaliana* in transgenic potato plants of the Désirée variety. Désirée: non-transgenic starting variety Désirée as a control. DK1: transgenic control line var. Désirée only containing the Npt II gene under control of the NOS promoter.

Figure 2

Figure 2 shows the DNA sequences of the primers used and an svFv antibody used according to the invention.

The invention is explained by the following examples.

Example 1:   Increase in the expression of Npt II in transgenic potato tubers

The gene constructs described in Tjaden *et al.* (*supra*) for "antisense" decrease ("MPB/aATPT") or "sense" increase ("MBP/sATPT") of the plastidiary ATP/ADP transporter activity in potato tubers were each ligated blunt-end into the opened and filled-in singular HindIII restriction site of the binary vector pSR 8-30 (*cf.* Düring *et al.*, *supra*; Porsch *et al.*, Plant Molecular Biology **37**

(1998), 581-585). The two transformation vectors pSR8-30/sATPT were obtained. These two expression vectors were used separately for the transformation of *E. coli* SM10. Transformants were mixed with *Agrobacterium GV 3101* and incubated at 28°C overnight. (Koncz and Schell, Mol. Gen. Genet. **204** (1986); 383-396, Kocz *et al.*, Proc. Natl. Acad. Sci. U.S.A., **84** (1987), 131-135). Selection was made on carbenicillin, the bla gene necessary for this purpose being available in the above expression vectors. Selected clones of *Agrobacterium tumefaciens* were applied onto detached leaves, cut several times at the middle rib, of potato plants cv. Désirée and the leaves were incubated at 20°C in the dark for 2 days. Thereafter, the agrobacteria were washed off and plant growth substances were added to the potato leaves, so that preferably shoots regenerated. Furthermore, non-transformed cells were killed in the potato leaves by the addition of kanamycin to the plant medium. Growing shoots were cut off and were allowed to grow roots in the medium without plant growth substances but with kanamycin. The further cultivation of the potato plants was performed as usual. On the one hand, transgenic lines including the antisense gene construct and, on the other hand, transgenic lines including the sense gene construct were obtained. The regenerated potato lines were planted in soil and grown in a greenhouse. After the ripening of the potato plants, the tubers were harvested and stored for the protein-chemical studies.

In order to study the antisense effect on the foreign gene expression, the NptII content in tuber extracts of all lines was compared by means of ELISA, since apart from Désirée all transgenic lines express the Npt II gene under the control of the nos promoter. The detection limit in the sandwich ELISA is at 0.5 ng Npt II/ml extract. The results show that the antisense lines MPB/aATPT/05, MPB/aATPT/13 and MPB/aATPT/22 contain Npt II concentrations which are 9 to 10 times as high as the content of the control. The determined Npt II values are about 0.7 ng/ml in the control DK1 and thus at the same order as those of the sense plants.

It showed that a major increase of the Npt II gene expression could be effected in transgenic potato tubers by using the described antisense constructs according to the invention.

Example 2:   Increase in the expression of scFv antibodies in transgenic potato tubers

For this test, the plants described in Example 1 were hyper-transformed with a gene construct which codes for an scFv antibody. The binary vector pLH9000Hyg was obtained by removing by means of restriction digest with XbaI and SpeI the kanamycin resistance-mediating expression cassette of the binary vector pLH9000 (L. Hausmann and R. Töpfer, *Vorträge Pflanzenzüchtung* [Lectures on Plant cultivation] **45** (1999) 155-172). In its place, a hygromycin resistance-mediating expression cassette was inserted which had been produced by amplification by PCR with primers

TCT AGA GAT CAT GAG CGG AGA ATT AA

and

ACT AGT AAT TCC CAT CTT GAA AGA AA

from the binary vector BinHygTOp (GenBank Gl:886843) and subsequent restriction digest using XbaI and SpeI. An expression cassette containing the gene for a single-chain (scFv) antibody having the sequence shown in figure 2 under the control of the CAMV 35S promoter was ligated into the opened SalI restriction site of the binary vector pLH9000Hyg. The transformation vector pLH9000Hyg/scFv was obtained.

This expression vector was used for the transformation of *E. coli* SM10. Transformants were mixed with agrobacterium GV 3101 and incubated at 28°C overnight (Koncz (*supra*)). Selection was made on streptomycin, the aadA gene necessary for this purpose being present in the above expression vectors. Selection clones of *Agrobacterium tumefaciens* were applied onto detached leaves, cut several times at the middle rib, of the potato plants described in Example 1, and the leaves were incubated at 20°C in the dark for 2 days. Thereafter, the agrobacteria were washed off and plant growth substances were added to the potato leaves, so that preferably shoots regenerated. Furthermore, non-transformed cells in the potato leaves were killed by the addition of hygromycin to the plant medium. Growing shoots were cut off and were allowed to root on

the medium without plant growth substances but with hygromycin. The potato plants were further cultivated as usual. T ransgenic lines including the antisense gene construct and the scFv gene construct, transgenic lines including the sense gene construct and the scFv gene constructs, and transgenic lines which only included the scFv gene construct were obtained. The regenerated potato lines were planted in soil and grown in a greenhouse. After the ripening of the plants, the resulting potato tubers were stored until the protein-chemical study was conducted.

In order to study the antisense effect on the foreign protein content, the content of scFv in extracts of potato tubers of all lines was compared by means of ELISA. The detection limit in the sandwich ELISA is at 500 ng scFv/ml extract. The results show that the antisense lines MPB/aATPT/05/scFv/05, MPB/aATPT/05/scFv/08 and MPB/aATPT/05/scFV/12 contain scFv concentrations which are 5 to 10 times as high as the content of the sense lines.

A major increase in the expression of the scFv gene in transgenic potato plants could be effected by using the described antisense constructs according to the invention.